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Legend for figures

Fig.1 Examples of volume of interest (VOI) overlaid on the average MRI of all subjects in stereotaxic space. Left: The VOI of the right caudate necleus. Middle: The VOI of the right putamen, Right: The VOI of the right ventral striatum.

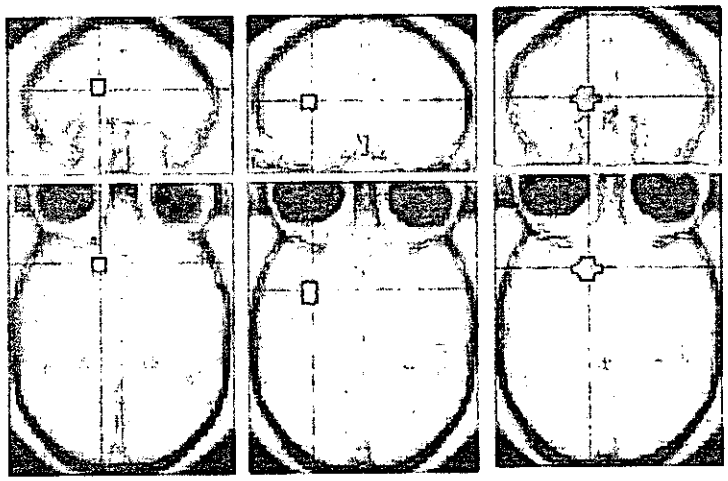

Fig.2 A SPM of the change in [11C] raclopride binding potential (BP) overlaid on the the average MRI. A siginificantly decreased [11C] raclopride BP is noted in the bilateral ventral striatum including the nucleus accumbens (NAc) compared with rTMS of the sham condition.

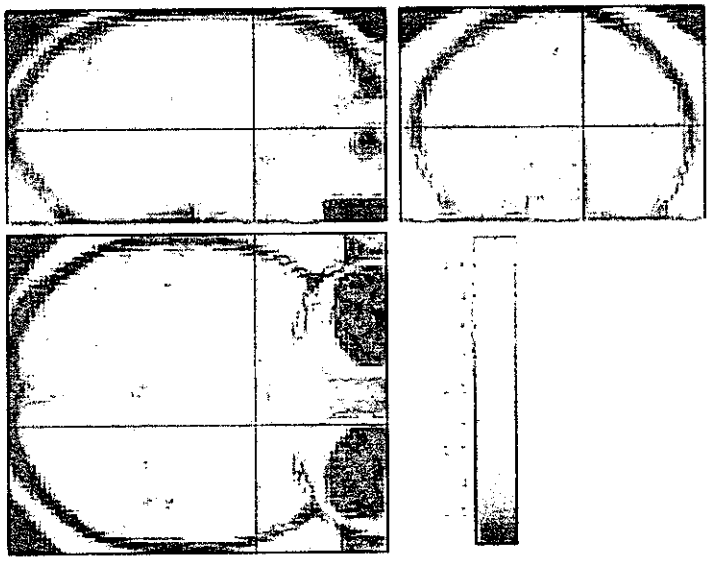

Fig.3 A SPM of the change in [11C] raclopride binding potential (BP) overlaid on the the average MRI. A siginificantly increased [11C] raclopride BP is noted in the posterior lateral part of the right putamen compared with rTMS of the sham condition.

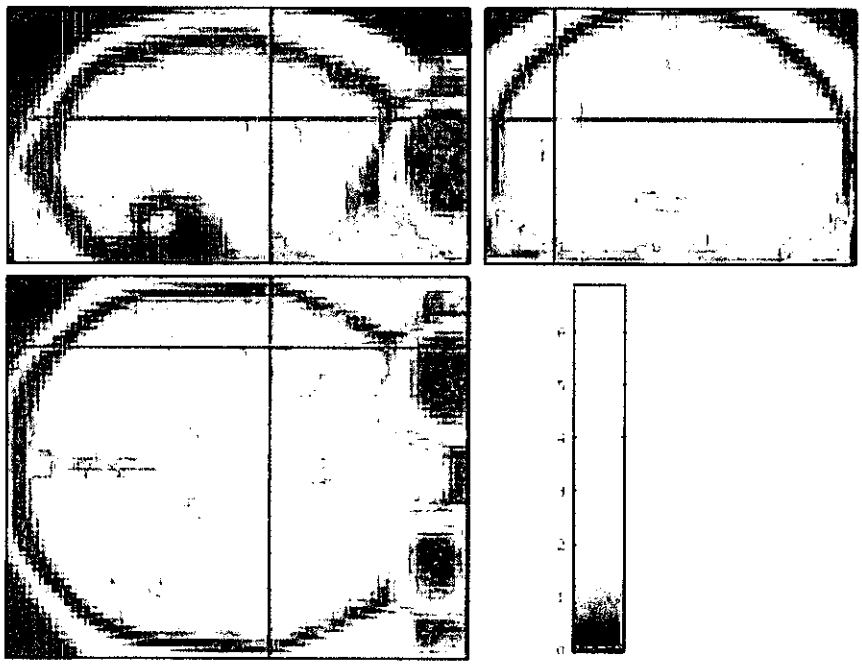

Table 1 Physiological data during sham stimulation and rTMS

	Blood presser		Pulse	PaO2	PaCo2	Bispectral index
	systolic	diastolic				
Sham	136.5 (19.1)	90.3(14.3)	133.9 (16.0)	127.2 (14.9)	38.0 (1.6)	86.8 (3.7)
rTMS	140.5 (18.8)	92.5 (19.4)	139.3 (15.2)	127.3 (19.0)	38.3 (1.7)	87.8 (3.7)

Table 2 C-11 raclopride binding potential in the striatum after rTMS and sham stimulation

	BP after sham	BP after rTMS	magnitude of change	p value
	mean (S.D.)	mean (S.D.)	mean (S.D.) (%)	for Wilcoxon test
Ventral striatum				
R	2.605 (0.074)	2.413 (0.13)	-8.12 (4.91)	0.0117
L	2.635(0.154)	2.436 (0.07)	-8.2 (5.77)	0.0117
Putamen				
R	1.993 (0.191)	2.22 (0.232)	10.51(2.98)	0.0117
L	1.730(0.198)	1.690 (0.113)	-2.07 (7.58)	0.6744
Caudate necleus				
R	2.542 (0.056)	2.648 (0.115)	3.82 (6.8876)	0.0929
L	2.712 (0.118)	2.636 (0.135)	-3.15 (7.615)	0.1614

Regional cerebral blood flow changes during prefrontal transcranial magnetic stimulation: A ^{15}O-labeled H_2O PET study

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Summary: Recently, repetitive TMS (rTMS) has been used as a potential treatment for neuropsychiatric disorders. The most successful example of therapeutic rTMS is an application to treatment of depression. Several studies have shown antidepressant effects of rapid rTMS over the left dorsolateral prefrontal cortex (DLPFC), whereas some studies suggested the effectiveness of slow rTMS over the right DLPFC. Despite the growing interest in therapeutic application of rTMS, it has several methodological issues to be resolved. Further, the precise mechanisms of action of rTMS over the DLPFC for an antidepressant are still unknown. To clarify these mechanisms of slow rTMS over the right prefrontal cortex, we measured regional cerebral blood flow (rCBF) during sham, during rTMS and after stimulation using repeated ^{15}O-labeled H_2O PET scanning in seven healthy subjects. We found that the slow rTMS over the right DLPFC could produce significant rCBF increase in the ipsilateral anterior cingulate cortex (ACC) including rostral ACC and neighboring medial prefrontal cortex during stimulation as compared with sham stimulation. On the other hand, rCBF decrease was noted in the contralateral cuneus. The lasting activation was noted in the ipsilateral medial prefrontal cortex, contralateral ventrolateral PFC, and the contralateral ventral striatum including the nucleus accumbens. These data indicate that slow rTMS is able to produce rCBF changes in the distant brain areas including paralimbic system and frontal cortex. We conclude that such rCBF changes should explain antidepressant effects of rTMS. Particularly, the lasting effect on the ventral striatum should reflect modulatory effects of rTMS over the DLPFC on meso-limbic dopaminergic system, which must play critical role in antidepressant effects.

Key words: PET, rTMS, depression, dopamine

Introduction

Transcranial magnetic stimulation (TMS) has been used as a research tool for brain mapping. During TMS, an electrical current passes through a wire coil placed over the scalp. This current induces an electrical field in the brain that produces a depolarization of nerve cells resulting in the stimulation or disruption of brain activity. Recently, repetitive TMS (rTMS) has also been investigated as a potential treatment for neuropsychiatric disorders such as depression (Pascal Leone et al., 1996, George et al., 1997, Klein et al., 1999, Berman et al., 2000), schizophrenia (George et al. 1999), and Parkinson disease (Dragasevic et al., 2002). The most successful example of experimental therapeutic rTMS is its use for depression. Following observation that rTMS might affect mood in normal subjects (Bickford et al., 1987), investigators have applied rTMS in patients with depression. Several studies have indicated that rapid rTMS (10-20Hz) applied to the left dorsolateral prefrontal cortex (DLPFC) may improve depression (George et al., 1995, Pascal Leone et al., 1996, George et al., 1997, Berman et al., 2000). Conversely, slow rTMS (1Hz) delivered to the right DLPFC may also improve depression (Klein et al., 1999). Despite the growing interest in therapeutic application of rTMS, several methodological issues remain to be solved; what kind of sham stimulation is appropriate, or what stimulation parameters should be used such as sites of stimulation, intensity, rate, train length and number of trains so on (George et al., 1999, Okabe et al. 2002b).

Precise mechanisms of action of rTMS over the DLPFC as an antidepressant are still unknown. To data, most of the basic information about neurobiological effects of TMS in humans comes from electrophysiological studies over the motor and visual cortex (Amassian et al., 1993, Ziemann and Hallett, 2000). Unlike motor and visual cortices, the DLPFC has no good electrophysiological markers to evaluate its function and has a different cytoarchitecture from those of primary sensorimotor areas. Therefore, neuroimaging studies have been used to clarify neurophysiologic effects of rTMS administration over the DLPFC in terms of hemodynamic and metabolic changes (George et al., 1995, Kimbrel et al., 1999, George et al., 1999a, 199b, Nahas et

al., 2001a, 2001b, Kimbrel et al., 2002). Although, most of studies demonstrated that rTMS over the DLPFC produced changes of cerebral activity in the local and distant areas, results have some inconsistency. As well as clinical therapeutic trial, neuroimaging studies have methodological issues involving not only parameters of stimulation, but also imaging modalities. Furthermore, most of them study effects of high frequency stimulation over the left DLPFC. As far as we know, no neuroimaging studies have been done on the effects of slow rTMS over the right DLPFC that has also antidepressant effects. To clarify neurophysiologic effects of slow rTMS over the right DLPFC, we measured regional cerebral blood flow (rCBF) during sham, rTMS over the right DLPFC (1Hz, 100% motor threshold) and after stimulation using repeated ^{15}O-labeled H_2O PET scanning in normal subjects.

METHODS

1. Experimental design

In seven healthy volunteers, a figure-eight TMS coil was positioned over the right DLPFC, and rCBF was measured in nine 90 sec ^{15}O-labeled H_2O scans acquired with the CTI/Siemens EXCT tomograph with an interval of 10 min between each scan. During all scans the subjects relaxed and kept their eyes closed. Fig. 1 demonstrates the schema of our protocol. The subjects were scanned under the following three conditions: 1) sham stimulation with no rTMS (before rTMS); 2) real stimulation over the right DLPFC (100 trains with 1Hz at 100% motor threshold); and 3) sham stimulation with no rTMS (after rTMS). In each subject, scanning was repeated three times in each of three conditions. The order of scans was not randomized, because rTMS have lasting effects. Between each scan during real stimulation, subjects were received rTMS. During PET sessions, a train of 100 stimuli was given over the right DLPFC eight times in each subject.

2. Subjects

Seven male subjects volunteered to participate in the study after giving written informed consent (mean age =38 years-old, sd= 6.5). All subjects were right-handed. Following the safety guidelines for the use of a rapid rate TMS in normal volunteers (Pascual-Leone et al., 1993 Wassermann 1998), we screened the subjects for a history of neurological disorders, in particular a personal and family history of epilepsy. The study was approved by the Research and Ethics Committee of the National Center Hospital of Mental, Nervous and Muscular Disorders, National Center of Neurology and Psychiatry.

2. TMS procedure

TMS was performed with a magnetic stimulator AAA-15486 (Nihon Kohden Co, Tokyo, Japan) equipped with a figure 8-coil over the right DLPFC. Subjects received 8 trains of 100 stimuli at 1Hz (100% motor threshold [MT]). The MT was determined by placing the coil over the right primary motor cortex and measuring the minimum intensity of stimulation required to elicit a visible movement at rest in the left first dorsal interosseous (FDI) muscle. The right DLPFC

stimulation site was defined as the location 5 cm rostal to the hand motor area, namely the site of stimulation for FDI. Once the coil location was determined, the coil was fixed there with a plastic arm throughout the experiment to avoid any movement of the coil during scans (fig.2).

3. PET acquisition

PET scans were obtained with a CTI/Siemens EXCT, 47-slice tomograph operated in a 3-D acquisition mode. The distribution of CBF was measured during a 90-s scan by means of the ^{15}O-labeled H_2O bolus method (Raichel et al., 1983). In each scan, 7mCi of ^{15}O-labeled H_2O were injected into the right antecubital vein. The CBF images were reconstructed with a 14-mm Hanning filter. Once the subject and the coil assembly were positioned in the scanner, a 6-min transmission scan was performed. The transmission data were used to correct for attenuation of gamma rays due to all objects in the scanner, including the coil and the coil mount. During rTMS condition, we began rTMS (1Hz, 100 trains) 10 sec before the start of an intravenous injection of ^{15}O-labeled H_2O. To exclude other components of rTMS than currents in the brain, in sham stimulation, we gave sampled rTMS sound from a speaker behind the subject's head. We began to give rTMS sound at a rate of 1Hz just the same as a real stimulation 10 sec prior to the start of intravenous injection. We performed unshielded concurrent TMS/PET in the real stimulation condition, and it did not have a serious influence on the image quality in our scanner. This fact is consistent with a recent report by Narayana et al.(2002) that unshielded concurrent TMS/PET did not adversely affect the image quality in their PET scanner (Narayana et al., 2002).

4. Image analysis

Data were analyzed with Statistical Parametric Mapping software (SPM99, http//:www.fil.ion.ucl.ac.uk/spm). Scans were realigned and spatially normalized to the standard stereotactic space of Talairach using PET template (Talairach J and Tournoux P., 1988). The parameter for affine and quadratic transformation to the PET template that was already fit for Talairach space was estimated by least-squares means. Data were then smoothed in a spatial domain (full width at half-maxim = 10 x 10 x 10 mm) to improve the signal to noise ratio. After specifying

the appropriate design matrix, the condition and subject effects were estimated according to the general linear model. Global normalization was performed using subject's specific analysis of covariance. To test hypotheses about regionally specific condition effects, the estimates were compared by means of linear contrasts of each sham, stimulation and lasting condition. The resulting set of voxel values for each contrast constituted a statistical parametric map of the t statistic SPM {t}(p = 0.001 with correction for multiple comparison). Some of previous studies demonstrated rCBF or glucose metabolic changes under the coil. To avoid a type II error, therefore, we also applied lesser rigorous statistical threshold (SPM {F}, p = 0.001, no corrections for multiple comparisons) in the analysis of rCBF changes at the right DLPFC.

5. Estimation of the coil location

The coil location in each subject was confirmed retrospectively by using transmission and emission images and MR image. Firstly, emission scan images were co registered to T1 weighted volume MR images using the function of coregistration in SPM99, and then transmission images were co-registered to MR images using the same parameters for coregistration emission images to MR images. Secondary, using MRIcro (http//www.mricro.com) co-registered transmission images having information of the coil location were superimposed on MR images (Fig.3). Finally, MR images were spatially normalized to the standard stereotactic space of Talairach using a T1 MR mage template of SPM99 and stimulated areas were evaluated based on the Talairach coordinate system (Talairach J and Tournoux P., 1988). This procedure allows us to estimate precise locations of the coil during PET scanning on the standardized anatomical space.

Results

1. Coil position, Safety and Tolerability

None of the subjects reported any adverse effects. The mood changes were not found in any subjects. The coil was positioned over either of the right DLPFC, BA9 or BA46 in all the subjects (Table 1). The mean location of stimulated site was BA9 (Talairach coordination: x:y:z = 33.4, 34.1, 33.1).

2. rCBF changes associated with rTMS over the right DLPFC

During TMS compared to the sham before stimulation, there was a cluster of significantly increased rCBF in the right anterior cingulate cortex (ACC: BA32) extending the rostral ACC (BA24a) and medial prefrontal gyrus (BA10) (Table 2, Fig.4a and 4b). On the other hand, significantly decreased rCBF was noted in the left cuneus (fig.5). Of particular note, even with lesser rigorous statistical threshold (SPM {F}, p = 0.001 without correction for multiple comparison), there was no significant rCBF change from sham at the site of stimulation immediately underneath the coil.

We evaluated after effects of rTMS by comparing the conditions before and after stimulation. Increased rCBF after stimulation was found in the left ventral striatum (ventral part of the putamen and nucleus accumbens [NAc]), ventral prefrontal areas (BA45, 47) and right medial prefrontal cortex (BA10) (Fig.6a, 6b, 6c). The lasting rCBF decrease was observed in the left cuneus with lesser rigorous statistical threshold (p = 0.001, without correction for multiple comparison), however, no survived clusters were found after correction for multiple comparison.

Discussion

The combining rTMS and repeated ^{15}O-labeled H_2O PET have been applied to investigate functional connectivity between the areas under the coil and distant areas (Paus et al., 1997, Fox et al.1997). To date, this technique revealed functional connectivity of the motor or eye movement systems in humans (Paus et al., 1997, Fox et al.1997). This is the first prefrontal study using repeated ^{15}O-labeled H_2O PET, which allow us to evaluate not only rCBF changes during rTMS but also after effects. Furthermore, unlike previous prefrontal studies using FDG-PET or perfusion SPECT, our method provide a large number of observations that enables us to apply more rigorous statistical thresholds than the previous studies.

In the present study, we found that slow rTMS over the right DLPFC activated the ipsilateral ACC, whereas it produced rCBF decrease in the contralateral cuneus (BA18). The after effects were noted as activations in the ipsilateral medial prefrontal cortex (BA9), contralateral

ventrolateral prefrontal cortex (BA45, 47), and the ventral striatum including the nucleus accumbens (NAc). Generally, previous neuroimaging studies combined with rapid rTMS have suggested that prefrontal rTMS has local cortical effects immediately below the site of stimulation and secondary limbic changes as a distant effect (George et al., 1995, Kimbrel et al., 1999, George et al., 1999a, 199b., Teneback et al., 1999, Nahas et al., 2001a, 2001b, Kimbrel et al., 2002). Although our study failed to demonstrate local cortical effects, it demonstrated slow rTMS over the DLPFC could produce rCBF changes in the distant paralimbic system and other areas involving in emotional process, such as ACC and NAc.

We first will discuss rCBF changes in distant areas induced by stimulation, and then discuss lack of local changes in this study.

In our study, activation during rTMS was observed in the ACC (BA24a: rostral cingulate cortex, BA32: paralimbic cingulate cortex) and adjutant medial prefrontal area, BA10. The intrinsic connections of the ACC with other frontal cortical areas are not limited to immediate neighbors, but also reach more distant areas, particularly, DLPFC (Barbas and Pandya, 1989). The ACC activation in this study can be explained by such a direct cortico-cortical connectivity between the ACC and DLPFC. The ACC has been shown to be blunted in depressed subjects undergoing a neuropsychological challenge. Using perfusion SPECT, Teneback et al. (1999) demonstrated that in limbic and paralimbic regions including the ACC (BA32), both rTMS and placebo effects evoked rCBF changes as a function of mood improvement. The fact suggests a possibility that the observed ACC activity change reflects an emotional change from depression. In our study, however, no subjects showed any mood changes after rTMS and the location of the ACC activation was more ventral area to that in their study (Teneback et al., 1999). Based on these, we conclude that activation at ACC is not due to an emotional changes induced by rTMS in our subjects. The human ACC has been considered to be involved in emotion, attention, and motor control (Paus, 2001). The rostral cingulate (BA24a) and other prosiocortical areas (BA24b, BA25) are known to receive projections from the amygdala and from the ventral striatum, therefore, their activity can be

influenced by emotional and motivational states (Paus, 2001). On the basis of results of pharmacological antidepressant therapy and neuroimaging studies in depressed subjects, Mayberg et al. hypothesized that the BA24a may serve an important regulatory role in overall network by facilitating the interaction between the ventral components consisting of limbic, paralimbic, and subcortical regions known to mediate circadian and vegetative aspects of depression, and the dorsal components (neocortical and superior limbic elements) hypothesized to mediate cognitive aspects of depression. We consider, therefore, the rostral ACC activation should have some relation with the therapeutic mechanisms of rTMS over DLPFC for depression that may be similar to those of antidepressant drug therapy.

Importantly, we found lasting activation in the contralateral ventral striatum including the nucleus accumbens (NAc). The ventral striatum is a part of limbic loop of basal ganglia, and the NAc has connections with prefrontal cortex and limbic system, such as amygdale, hippocampus, and orbito-frontal cortex, further, the rostral ACC (BA24a) receives projections from the ventral striatum. The ventral striatum has been implicated as a critical neuroanatomical substrate for the anticipation of rewards in mammals (Ikemoto and Panksepp, 1999). Several electrophysiological studies of monkeys indicate that dopaminergic projections from the ventral tegmental area of the midbrain to the NAc fire selectively in response to presentation of reward cues (Schultz et al., 1992). In human, the NAc is also considered to be associated with anticipation and motivation. Using fMRI, Knutson et al. reported the NAc codes for expected positive incentive value and its activity was correlated with self-reported happiness. They interpreted that increased NAc activation may be associated with dopamine release, because NAc dopamine release can produce hemodynamic changes in rats' brain (Marota et al., 2000). In addition, PET studies have demonstrated positive correlations between stimulant-induced dopamine release in the ventral striatum and ratings of euphoria in humans (Volkow et al., 1999; Drevets et al., 2001). We consider that the lasting effects in the ventral striatum should play an important role in antidepressant effects of rTMS over the DLPFC through mesolimbic dopaminergic system. Recently, Strafellea et al. reported that a

reduction in ^{11}C raclopride binding to dopamine receptors in the left dorsal caudate nucleus was observed in 8 volunteers after left DLPFC rTMS (Strafellea, et al. 2001). An animal study using intracerebral microdialysis also demonstrated that dorsal hippocampus, the shell of the nucleus accumbens and the dorsal striatum the extracellular concentration of dopamine was significantly elevated in response to rTMS (Keck M, 2002). These data imply that rTMS of frontal cortex has a modulatory effect on both the mesolimbic and the mesostriatal dopaminergic systems. This increase in dopaminergic neurotransmission may contribute to the therapeutic effects of rTMS in depression. The present lasting activation of NAc is consistent with the idea that the increment of dopaminergic neurotransmission should cause improvement of depression.

Amongst previous studies, the most consistent finding is local cortical effects immediately below the site of stimulation. The idea of DLPFC rTMS based upon the evidence of a link between the response to electroconvulsive therapy and changes in prefrontal function as well as neuroimaging studies reporting abnormalities in the PFC in patients with depression (George et al, 1994, Nobler and Sackheim, 1998, Drevets, 2000). In this context, the lack of changes in the DLPFC in this study seems to be somewhat unreasonable. Several factors must explain why we could not elicit any significant changes in the DLPFC under the coil. The most important factor is parameters of stimulation, particularly intensity of rTMS. Recently, Nahas et al. reported intensity dependent hemodynamic changes using 1Hz rTMS (80%, 100%, and 120% MTs) over the left DLPFC combining BOLD MR imaging. According to their study, higher intensity stimulation produced greater local and distant activation and only 120% RMT rTMS could produce significant activation in the stimulated area. Further, they reported that greater effects were noted in distant areas than the stimulated areas at all intensities. In spite of different modalities for the measurement of hemodynamic changes, their results are compatible with our observation of the distant activation without activation in the stimulated area. RCBF changes at distant areas without any changes under the coil have also been seen in rTMS over the motor cortex (Okabe et al., 2002b in press). It is not surprising that rCBF changes occurred in some distant areas with no changes at the

right DLPFC in our study. Another point we should consider is how to determine the intensity in DLPFC stimulation. In most of clinical trials using rTMS over the DLPFC, the intensity was usually determined by comparing with the MT. However, the threshold for DLPFC may differ from that for the motor cortex because of different distance from the skull or intrinsic cytoarchitecture between them. The underestimation of the threshold for DLPFC may cause to give a weak stimulation on DLPFC and show rCBF changes at only distant areas.

Another imperative factor we should consider is the mode of neuronal activation by rTMS. TMS should induce neuroaxonal depolarization and produce action potentials. This process dose not includes much synaptic activity. Therefore, rTMS may affect spiking outputs, but may not be necessarily associated with blood flow increases. This happened in rTMS over M1 (Baudewig et al., 2001). They showed that, in M1 under the coil, no rCBF changes were evoked by rTMS at an intensity of 0.9 RMT that should have activated the motor cortex. This dissociation between rCBF and neuronal activation must partly explain no CBF changes at the stimulated area.

Unexpectedly, we found rCBF decrease in the left cuneus during rTMS over the right DLPFC. Because direct connection between DLPFC and cuneus is not present, we have no good interpretation of it. One possible explanation is that rCBF change in the cuneus might be caused by activation of the frontal eye field (FEF: BA8) by spreaded currents because it is adjacent to the stimulated area (DLPFC: BA9). Paus et al. reported functional connectivity between FEF and cuneus using ^{15}O-labeled H_2O PET and rTMS technique (Paus et al., 1997). Their results support our conjecture.

There are some methodological limitations in this study. Firstly, the scan order was not randomized because of postulated lasting effect of rTMS. Secondly, during sham condition, we gave only sound but did not give local sensation of the head due to activation of cutaneous and muscle nerves (Okabe et al., in press). They may be one of confounding factors such as time effect, local sensation, discomfort and tiredness due to long time for PET procedure (although subjects did not complain discomfort or tiredness). Our results might be affected by non-specific brain activities